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Acute HIV infection: the impact of anti-retroviral treatment on cellular immune responses

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Summary

The overall value of initiating anti-retroviral therapy during the acute phase of human immunodeficiency virus type 1 (HIV-1) infection remains unclear. From a clinical perspective, the lack of data from controlled randomized clinical trials limits understanding of long-term effects of treatment on the clinical course of HIV infection. Based on available data, the impact of anti-retroviral therapy during acute infection on the immune response against HIV-1 is not particularly encouraging. Recent observations on the very early depletion of lymphocyte reservoirs in the gastrointestinal tract may partially explain the limited benefit of anti-retroviral therapy initiated during the acute phase of HIV-1 infection. This may also help to explain the dichotomy between early observations demonstrating apparent immunological benefit with early anti-retroviral treatment that were associated none the less with inability to control viral replication following treatment interruption.

Keywords: acute infection, anti-retroviral therapy, cellular mediated immunity, HIV-1, immune reconstitution

Introduction

Human immunodeficiency virus type 1 (HIV-1) can infect exposed individuals through mucosal surfaces or due to direct inoculation into the bloodstream. The virus subsequently infects $CD4^+$ T lymphocytes directly or as a consequence of their interaction with virus-bearing dendritic cells in the lymphoid tissues [1,2]. As with many other viral infections, the onset of clinical manifestations in HIV-infected individuals is usually observed 2–4 weeks after exposure [3]. It has been reported that up to 90% of patients with acute HIV-1 infection (AHI) report to a physician due to the presence of symptoms that are most often described as a flu-like syndrome. The symptoms and signs seen in association with acute HIV infection may include fever, lymphadenopathy, pharyngitis, mucocutaneous lesions, myalgia/arthralgia, headache, gastrointestinal symptoms (diarrhoea, nausea/vomiting) and weight loss [4]. Notably, none of these findings can be considered specific, although their persistence for periods as long as 10 weeks and the presence of mucocutaneous ulcers are suggestive of AHI [5]. A particular challenge in diagnosing AHI is the fact that the onset of symptoms coincides typically with peak HIV viraemia [6], and usually precedes by 2–3 weeks the development of HIV-specific antibodies that are necessary for diagnosis using routine HIV screening tests [7]. Therefore, the diagnosis of AHI is often quite delayed (as long as 8–12 weeks after infection has occurred), and this delay probably has a great impact on the damage that occurs to the immune system before the initiation of anti-retroviral therapy (ART). In addition, delays in the diagnosis of AHI also prevent the implementation of secondary prevention measures with a group of patients who are at high risk of transmitting HIV during unprotected sexual encounters, due at least in part to the high levels of viraemia characteristic of this stage of infection [8,9].

Once the diagnosis has been made, additional challenges include the absence of clear guidelines on whether to initiate anti-retroviral treatment at this stage and the selection of an initial regimen if treatment is elected. Published guidelines for anti-retroviral therapy specifically note the lack of consensus in this area. The US Department of Health and Human Services (DHHS) guidelines (available at http://aidsinfo.nih.gov/contentfiles/AdultandAdolescentGL.pdf), developed by the working group of the office of AIDS

Research Advisory Council, and International AIDS Society (IAS)–USA guidelines [10] are appropriately vague, because of insufficient data to establish clear benefits of ART in acute infection [11]. The British HIV Association (BHIVA) recommends treatment for people with AHI for relief of symptoms only. As a result, the decision to initiate ART is left to the individual AHI patient and his or her health care provider. Common laboratory parameters used to guide treatment decisions for people with chronic HIV infection (such as HIV RNA levels and CD4 counts) are not clearly of value for acutely infected patients. As for choice of treatment regimen, most available data is on the use of standard-of-care regimens for chronic HIV infection, i.e. recommended first-line regimens for treatment-naive patients as outlined in IAS–USA as well as BHIVA guidelines [10].

These various considerations illustrate the clinical challenges associated with diagnosing AHI and initiating ART treatment in a timely manner using an appropriate drug regimen. Bearing these factors in mind, we will discuss what is known of the impact current ART initiated during AHI may have on the reconstitution of cellular-mediated immune responses.

Impact of ART on cellular immune responses

The ability of HIV-1 to infect and to deplete the $CD4^+$ T lymphocyte subset has been considered for years to be the hallmark of the pathogenesis of the acquired immunodeficiency syndrome (AIDS) [12]. The ongoing depletion of this pool of cells is thought to be the main cause of the progressive impairment of the adaptive and innate immune responses, the inability to control virus replication and the onset of opportunistic infections and other AIDS-related clinical manifestations.

Several studies have demonstrated that cellular-mediated immune responses may play a central role in controlling the initial burst of virus replication [13–15]. For years, $CD8^+$ T cell responses have been considered the main line of defence during acute HIV infection and have been associated with delayed disease progression [16].

Early observations by Rosenberg and collaborators in a cohort of 10 untreated acutely infected individuals suggested that detectable $CD4^+$ T cell responses directed mainly against the Gag p24 antigenic region were correlated inversely with subsequent virus load [17]. Interestingly, the study reported that three individuals who started ART at the time of AHI diagnosis and before seroconversion were also able to develop a strong anti-p24 lymphoproliferative response in synchrony with a decrease in viral load. This was in striking contrast with previous observations showing that chronically infected individuals lack strong lymphoproliferative responses to HIV-1 antigens, despite suppressive antiretroviral therapy [18,19]. Subsequently, one study compared the magnitude and breath of T cell responses in three groups of individuals: (1) those receiving ART pre-seroconversion ($n = 19$); (2) those treated post-seroconversion and within 180 days of infection ($n = 11$); and (3) those treated during chronic infection more than 180 days after infection ($n = 10$) [20]. After receiving ART for 1 years, the pre-seroconversion group had the highest significant detectable CD4 lymphoproliferative responses [stimulation index (SI) = 43 ± 13] compared to either the post-seroconversion ($SI = 29 \pm 10$) or the chronically infected groups ($SI = 8 \pm 3$). Oxenius and collaborators observed similar beneficial effects from the initiation of ART during acute infection on the $CD4^+$ T cell compartment. Their data indicated that the frequency of interferon (IFN)-γ-secreting $CD4^+$ T cells was elevated in six patients up to 36 months post-treatment and directed mainly against HIV-1 Env and Gag antigens [21]. Moreover, Cossarizza and colleagues studied 18 individuals who received therapy during AHI for 1 year and compared the profile of their $CD4^+$ Vβ T cell receptor repertoire to that observed in ART-treated chronically infected individuals and healthy non-infected donors [22]. The clonality of the $CD4^+$ T cell population was apparently restored in AHI patients, but not in the chronically infected individuals. These findings, again, suggested a beneficial impact of ART initiated during AHI on the $CD4^+$ T cell population.

Several studies have also reported that the initiation of ART during acute infection can boost $CD8^+$ T cell responses [20,21,23–25]. These data suggested that $CD8^+$ T cell responses were preserved by the initiation of therapy during AHI. However, the frequency and breadth of cytotoxic and IFN-γ-secreting $CD8^+$ T cells decreased over time with suppression of viraemia, probably as a result of the lower antigen stimulation. Another encouraging observation was that the virus diversification in patients treated during AHI was lower and therefore escape mutants had not been generated, despite the strong cellular-mediated immune responses [20].

Although the initial observations on the importance of ART initiation in preserving T cell responses in acute infection was demonstrated in patients treated prior to seroconversion [20], more recent studies suggest that ART may have similar positive effects if initiated during the first 90 days post-infection [25,26]. Furthermore, recent investigations by Younes and colleagues on the breadth and magnitude of $CD4^+$ T cell responses by lymphoproliferation and class-II-tetramer assays suggest that initiation of ART prior to seroconversion may impact negatively the maturation of HIV-specific IFN-γ-secreting $CD4^+$ T cells as a consequence of the limited time for interaction of antigen-presenting cells with virus due to low antigenaemia [26].

With respect to duration of therapy, Oxenius and collaborators suggested that even a short course of ART treatment initiated during AHI and continued for up to 24 weeks post-infection could exert beneficial effects on the longevity and breadth of $CD8^+$ and $CD4^+$ T cell responses [21].

Impact of T cell responses generated under AHI–ART on virus load

A consistent finding among individuals treated during AHI has been higher proliferative capacity and frequency and breadth of IFN-γ- and interleukin (IL)-2-secreting T cells in the groups receiving ART during AHI. This outcome was observed independently of the duration of therapy, ranging from 12 weeks [27] to 98 weeks [28]. Based on these findings and those outlined in the previous section, indicating that ART initiation within 90 days of infection preserved strong T cell responses, one can hypothesize that subsequent treatment interruption in these subjects might result in control of HIV infection. Although viral control was demonstrated in three of 14 individuals studied by Rosenberg and colleagues [17], subsequent studies did not observe consistent viral control following treatment interruption. Therefore, despite preservation of cellular immune responses, initiation of ART during AHI has not shown a consistent and significant impact on decreasing viral rebound following treatment interruption [27–30].

In summary, there are no clear data indicating that early initiation of ART during AHI lowers the viral set-point or delays disease progression following treatment interruption.

Controlling virus replication: a multi-faceted picture

The simplest potential reason for the inability to demonstrate control of virus replication following ART interruption in patients treated during AHI is that the focus on cellular-mediated immune responses as the key factor in controlling HIV-1 replication may be incorrect. Indeed, this relationship may not be central, and other components of the immune response against HIV-1, as well as genetic factors, could correlate with protection from infection and/or disease progression [31].

If an overly narrow focus on cellular responses is a limiting factor in understanding viral control, consideration of both humoral and innate immunity may be important. Both responses can be influenced positively by ART initiated during AHI [32,33], but these factors may still not be sufficient for control of virus replication following treatment interruption.

As for other factors, even large cohort studies have been unable to establish whether genetic factors, such as selected HLA alleles and killer inhibitory receptor (KIR) haplotypes, may contribute to the control of virus replication [34–36] or whether they may have synergistic effects with the initiation of ART during AHI.

ART-preserved cellular immunity: reasons for failure

Lack of a clear understanding regarding which factors are important in control of viral replication limits the ability to discern which parameters should be studied to assess the effect of ART initiated during AHI [31]. Consideration of data on T cell responses in individuals who control HIV-1 replication successfully without ART for up to 18 years post-infection is illuminating. Viral control in these 'long-term non-progressor' patients (LTNP) may not relate to a single factor such as proliferative capability and/or single cytokine production. Migueles and colleagues demonstrated that the preservation of proliferative capability and perforin production of $CD8^+$ T lymphocytes set LTNP apart from those who do not control virus replication [37]. Betts and collaborators recently reported a higher frequency and breadth of $CD8^+$ T cell responses with a multifunctional profile in LTNP compared with subjects who do not control virus replication successfully [38]. Both studies revealed that the quality of cellular-mediated immune responses may be more important than their mere frequency or breadth. One could argue that even these parameters simply reflect the preservation of $CD4^+$ T cell responses that sustain other cellular, humoral and innate components of the anti-viral responses. However, the question of how cellular immune responses are preserved in a limited number of infected subjects, and not in others, remains.

One hypothesis is that the immune responses involved in control of virus replication after treatment interruption may be compared to those described as a recall response after antigen-priming of the immune system, although virus replication and therefore antigen stimulation still occur during the latent phase of HIV-1 infection [12,39]. Janssen *et al*. demonstrated the importance of $CD4^+$ T cells providing the correct signal to the $CD8^+$ T cell compartment during the priming of the immune responses, as the recall $CD8^+$ T responses are impaired in the absence of this interaction [40]. In the course of HIV infection, the maturation and the functionality of $CD8^+$ T cells are impaired [41–45], reminders that the two fundamental effects of HIV infection on the immune system are severe depletion of $CD4^+$ T lymphocytes and persistent cellular activation. Accordingly, this raises the question: 'is ART initiated during AHI effective in preventing loss of $CD4^+$ T lymphocytes that may be one of the key factors in correctly priming the immune system?'

Loss of $CD4^+$ T cells during AHI

The preferential infection of anti-HIV-specific memory $CD4^+$ T cells during the course of HIV-1 infection has been well documented [46,47]. Surprisingly, it has also been shown that the $CCR5^+CD4^+$ lymphocytes that reside in the lamina propria of the lower gastrointestinal tract are the major targets for both simian immunodeficiency virus (SIV) and HIV-1 replication, independently of the route of infection [48–53]. These studies demonstrated that up to 60% of $CCR5^+CD4^+$ cells can be infected at the peak of

viraemia, and 80% are depleted within 4 weeks from infection as a consequence of direct virus replication [51] or apoptosis [52,53]. It is probably this early and massive depletion of $CD4^+$ T cell subsets that limits priming of the immune system and therefore control of HIV-1 infection during the early and late stages through functional cellular immune responses.

Recently, several groups have reported on the effect of ART initiated during AHI on reconstitution of mucosal lymphoid tissue. Results from these studies have been consistently disappointing and revealed a lack of reconstitution of lamina propria lymphocytes. In fact, Mehandru and collaborators studied 18 subjects, nine of whom started therapy pre-seroconversion and continued treatment for up to 7 years post-diagnosis [54]. Serial gastrointestinal biopsies were performed in a subset of these subjects at 1, 2 and 3 years' post-treatment initiation. Although reconstitution of $CD4^+$ T cells in the peripheral blood reached 67% at 3 years, the percentage of $CD4^+$ T cells in the mucosa of the gastrointestinal tract never exceeded 45% of the level observed in healthy individuals. Moreover, immunohistochemistry analysis of the biopsies revealed that the immune-inductive sites were fully reconstituted, whereas the effector sites of the lamina propria were persistently depleted. This partial reconstitution of the $CD4^+$ and $CD8^+$ T cell population in the mucosal tract confirmed what had previously been observed by Guadalupe and collaborators in a smaller number of individuals ($n = 3$) during the first 14 months of therapy initiated following seroconversion [55]. An additional interesting finding by Mehandru and his group was that activated $CD4^+$ and $CD8^+$ T cells were still elevated at the mucosal sites after 3–7 years of treatment, even in the presence of a minimal number of cells expressing viral mRNA. It has been postulated that the incomplete reconstitution of resident T cell populations in the lamina propria may allow a continuous leakage of foreign antigens from the lumen of the gastrointestinal tract that can persistently activate T cells and predispose them to apoptosis [54,56]. One premise behind the use of ART is that a high level of virus replication is the main reason for $CD4^+$ T cell depletion. However, it has been suggested recently that this may not be the case and that other co-factors may play an important role. For example, persistent antigenic stimulation may be a critical factor in the pathogenesis of damage to mucosal surfaces which, in turn, leads to apoptosis of lymphocytes [57]. In this situation, these secondary effects on the immune system cannot be addressed by ART therapy alone.

Taken together, these studies indicate that massive depletion of the T lymphocyte pool at the mucosal level takes place before it is possible to diagnose AHI in most patients, and certainly prior to initiation of ART. This lag probably impedes reconstitution or recovery of mucosal T cells, and could be the major factor preventing recovery from immunological damage sustained during peak HIV-1 replication. The inevitable consequence is, in most cases, that the immune system will not be able to control virus replication upon treatment interruption.

Clinical impact of ART

From a clinical perspective, the effect of ART on patient outcome may be more favourable overall than that observed in immunological studies. Hecht and collaborators detected a trend towards higher CD4 cell counts and lower HIV RNA levels among 13 people treated with ART during acute HIV infection when measured 24 weeks after ART interruption, if therapy was initiated within 2 weeks of seroconversion [27]. Initiation of therapy at a later time-point showed less benefit in 45 individuals when measured 72 weeks after treatment interruption. Moreover, there was a substantial decrease in virus load (approximating a two-thirds-$\log_{10}$ reduction) observed in the adjusted analysis at 72 weeks among those who were treated earlier, a reduction which corresponded to an approximate 50% reduction in the risk of HIV disease progression over 6 years [58].

Despite the fact that ART initiated during AHI may not consistently alter the viral set-point following treatment interruption, we need to consider other therapeutic benefits which may still justify its use: (1) control of symptoms related to acute retroviral syndrome; (2) reduction in viral load which probably decreases the risk of transmission; (3) potential slowing of the rate of disease progression among patients with a genetic predisposition; (4) decrease in the size of the latent HIV pool; (5) possible control of viraemia and disease progression during which time advances in therapeutic vaccines and other treatment modalities may evolve; and (6) possible slowing of the pace of CD4 cell count decline prior to institution of long-term ART.

Therefore, despite the fact that initiation of ART during AHI may not have a significant impact on immune reconstitution as currently understood, it may alter clinical outcomes for those diagnosed during acute HIV-1 infection. As noted by others, findings which could determine the most predictive immunological parameters for favourable clinical outcome have yet to be demonstrated in a randomized clinical trial (RCT) [59]. A randomized controlled trial of treatment *versus* no treatment in the setting of recent HIV infection is currently ongoing (ACTG 5217), but will not be able to address the more probable benefit of ART initiated during 'acute' infection, as most enrolled patients will be outside the acute infection time-frame by the time therapy is initiated. Given the challenges of conducting a large randomized study in patients with AHI, it is not likely that convincing data will be available for guideline development in the foreseeable future. Thus, the clinical use of ART during AHI will continue to be based on the best available evidence and expert opinion. As such, it should still be carefully evaluated on a case-by-case basis.

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